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Universitätsklinik für Kinder- und Jugendmedizin

Heidelberg, 26 March 2012

ERNDIM QA Scheme for qualitative urinary organic acid analysis

Annual Report 2011

Participation

The geographical distribution of the active participants of the quality assurance scheme organized and distributed through the centre of Heidelberg in 2011 is shown in Table 1. Sheffield and Heidelberg participate in each other's scheme and the two centers work closely together under the auspices of the ERNDIM Scientific Advisory Committee.

Table 1: Geographical distribution of participants				
Country	Number of laboratories		Country	Number of laboratories
Austria	3		Lithuania	1
Belgium	1		Luxembourg	1
Canada	6		Norway	1
Croatia	1		Philippines	1
Cyprus	1		Poland	2
Czech Republic	2		Slovakia	2
Denmark	1		Slovenia	1
Estonia	1		Spain	4
France	2		Sweden	2
Germany	13		Switzerland	3
Greece	1		The Netherlands	10
Hungary	1		Ukraine	1
India	1		United Kingdom	1
Italy	12		USA	11
Kingdom of Saudi Arabia	1			
Latvia	1			

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Samples and results

Three sets of three samples (total 9; sample number 187 --195) were distributed to 90 laboratories.

Table 2 shows number of returned results for each circulation and the number of late return.

Table 2: Receipt of results

Circulation	In time returns	Late returns	Total
1. circulation	83	4	87
2. circulation	87	0	87
3. circulation	85	1	86

Ninety-two percent of the participants returned results for all three circulations. Only one laboratory did not respond to any of the circulations (see also table 3)

Table 3: returned results

Circulations	Number of laboratories	%
3	83	92
2	5	6
1	1	1
0	1	1

Shipment of the samples

As the years before we sent out the samples for all three circulations together. This is only for organizational reasons especially to keep the costs for participating in this scheme as low as possible.

Please remember, the idea of the scheme is to measure the samples evenly spread over the year and report the results near the closing date!

Table 4: Distribution of scores for individual samples (laboratories making returns)

		-2	-1	0	1	2
Sample 187	Normal pattern					87
Sample 188	HMG-CoA-lyase deficiency				3	84
Sample 189	Propionic aciduria	1		1	1	84
Sample 190	Normal pattern			2		85
Sample 191	Citrullinaemia	4				83
Sample 192	Isovaleric aciduria	2				85
Sample 193	Normal pattern			2		84
Sample 194	3-methylcrotonylglycinuria	1			4	81
Sample 195	Tyrosinaemia type I	2		1	4	79

Scoring scheme

Individual returns for each sample were scored on the scale

2	Correct/satisfactory
1	helpful but incomplete
0	unhelpful
-2	misleading

The ERNDIM organisation is moving towards providing a single "Certificate" to cover participation and performance in all its schemes. The scheme organizers of the "Qualitative Organic Acid Scheme" in Sheffield and Heidelberg agreed on criteria to define "Participation" and "Satisfactory Performance".
We are aware that these criteria are rather arbitrary but we are convinced that they will represent the different contexts in which the participants are working.
So "Participation" will be defined as requiring at least two returns during a year and "Satisfactory Performance" as obtaining a score of 11 or more based on three returns (out of maximum 18). When two returns have been received a score of 7 or more (in this case possible maximum score 12) is satisfactory.

Comments on performance

Sample 187:

Patient details:	4-year-old girl with mental retardation
Known diagnosis:	Normal pattern
Overall Performance:	All participants reported a normal profile

Sample 188:

Patient details:	Neonate with muscular hypotonia, hypoglycemia and mild hyperammonaemia
Known diagnosis:	HMG-CoA-lyase deficiency

Analytical details:

The characteristic metabolites 3-methylglutaconic acid, 3-methylglutaric acid and 3-hydroxy-3-methylglutaric acid were identified by overall 96% of the participants. 3-Methylglutaconic acid as the most intensive metabolite was detected by 100%. 3-Hydroxy-3-methylglutaric acid was reported by 97% and 3-methylglutaric acid by 92% of the laboratories.

Minor metabolites that could also be seen in this urine sample were 3-hydroxyisovaleric acid and 3-methylcrotonylglycine. These were identified by 92% and 39% of the laboratories respectively.

Diagnosis:

HMG-CoA-lyase deficiency was diagnosed by 97% of the participants. 3% reported 3-methylglutaconic aciduria type I

Chromatogram

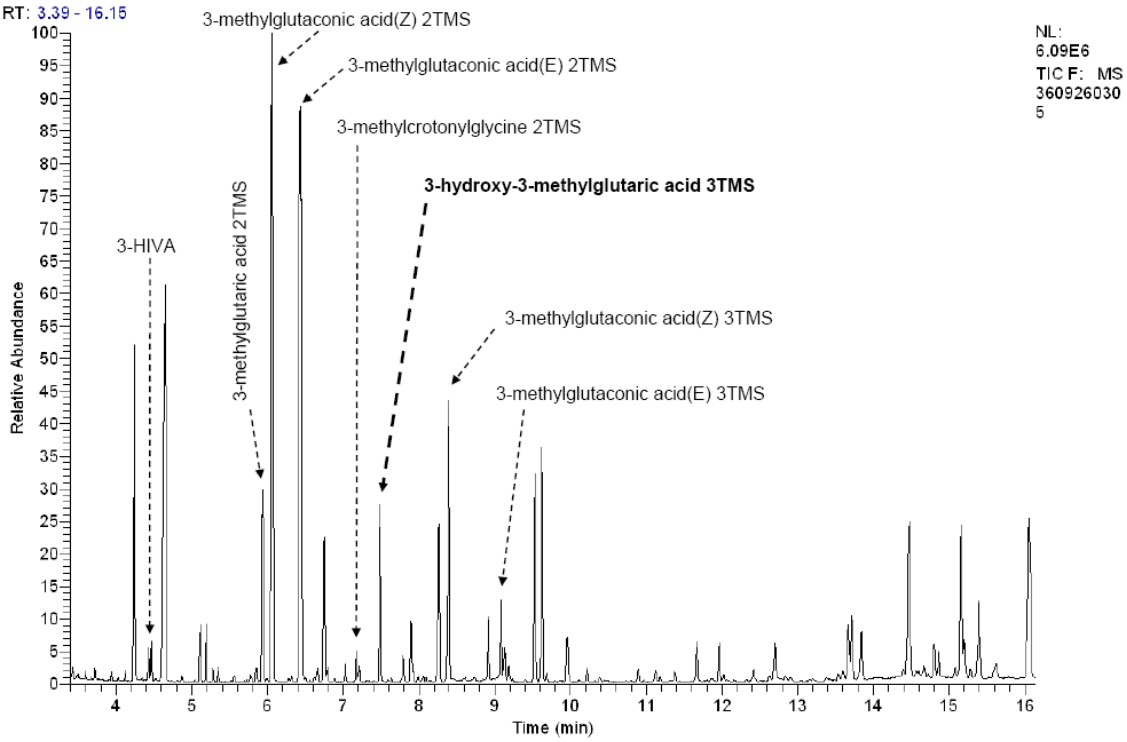

Fig 1: Organic acid profile of HMG-CoA-lyase deficiency

Sample 189:

Patient details: 6-month-old boy with acute encephalopathy

Known diagnosis: Propionic aciduria

Analytical details:

In this sample propionylglycine, 3-hydroxy-propionic acid and methylcitric acid can be identified. As trimethylsilyl derivatives both propionylglycine and methylcitric acid yield two signals thereby the signal for the propionylglycine diTMS is much more pronounced than the monoTMS derivative. Also detectable are the mono and di TMS derivative of tiglylglycine.

Overall 96% of the laboratories detected these metabolites whereas tiglylglycine was reported by only 71%.

Diagnosis:

Ninety-seven% of the participants diagnosed propionic aciduria.

Chromatogram:

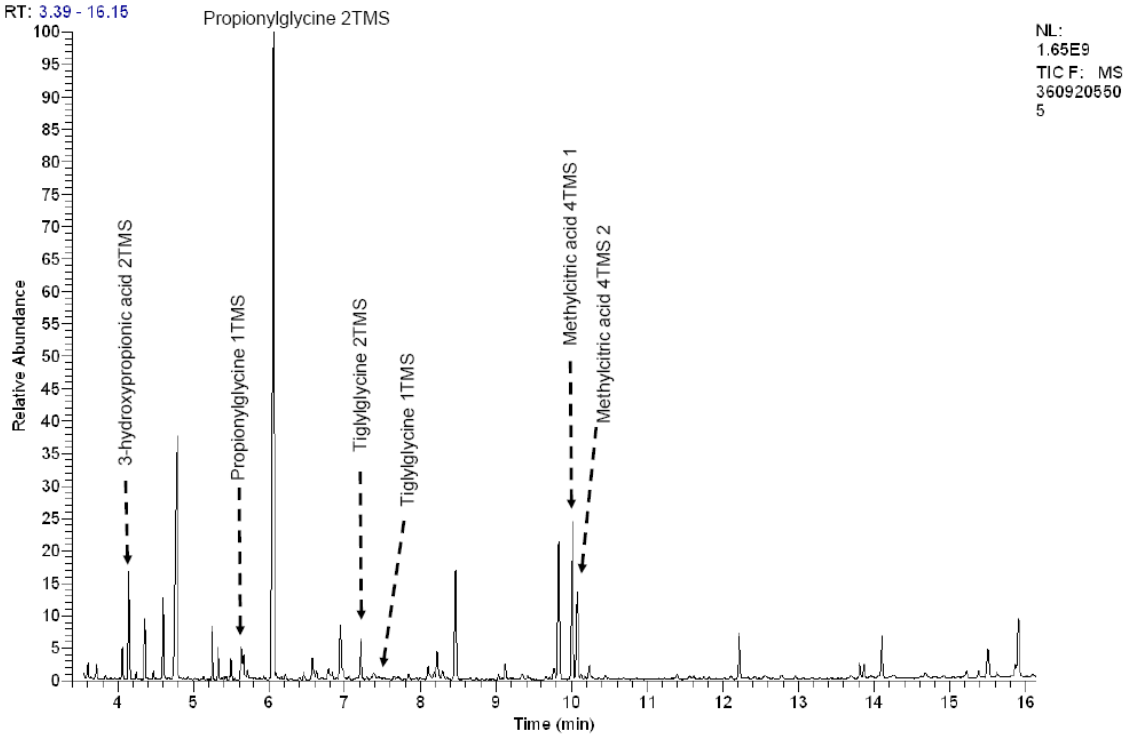

Fig 2: Organic acid profile of propionic aciduria

Sample 190:

Patient details: 13-month-old boy with developmental delay

Known diagnosis: Normal pattern

Overall Performance: Nearly all participants reported a normal profile

Sample 191:

Patient details: newborn boy with hypotonia, poor sucking and hyperammonaemia

Known diagnosis: Citrullinaemia

Analytical details:

Several increased metabolites could be detected in this sample. The chromatogram is dominated by a large peak of hippuric acid 2TMS and a minor peak of hippuric acid 1TMS. Furthermore lactic acid and 3-hydroxybutyric acid are seen as prominent signals. In addition the chromatogram shows moderate signals for the dicarboxylic acids adipic acid, suberic acid and sebacic acid in decreasing series.

Most important for the interpretation is the identification of orotic acid. 94% of the laboratories reported orotic acid.

Diagnosis:

Citrullinaemia could not be diagnosed from urinary organic acid analysis alone but needs the results from amino acid analysis showing increased citrulline. Therefore the accepted interpretation of the outcome of the organic acid analysis is orotic aciduria, OTC deficiency or urea cycle disorder in combination with the appropriate recommendations for further investigations.

Overall interpretative proficiency was 95%.

Chromatogram:

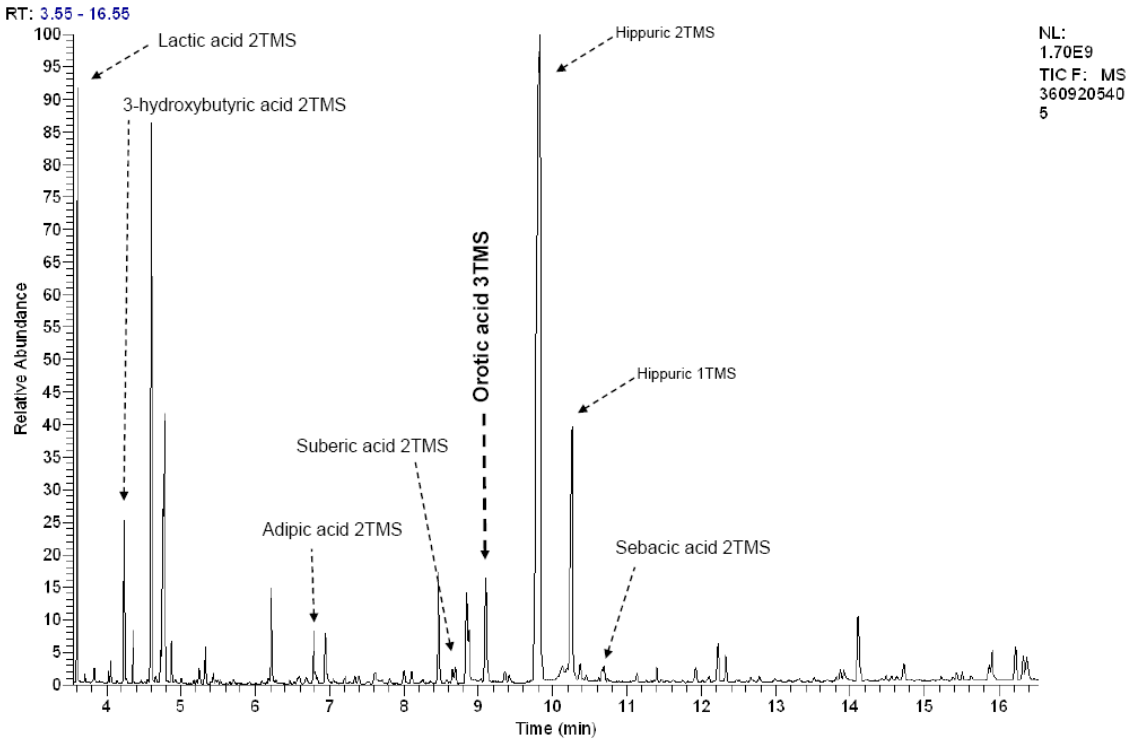

Fig 3: Organic acid profile of orotic aciduria

Sample 192:

Patient details: 13-year-old boy with acute acidosis

Known diagnosis: Isovaleric aciduria

Analytical details:

In the chromatogram two peaks for isovalerylglycine can be clearly detected with the highest intensity for the 2TMS derivative. 3-Hydroxyisovaleric acid is also detectable in low amounts. Isovalerylglycine was identified by 98% of the laboratories.

Diagnosis:

The diagnosis of isovaleric aciduria was given by 98% participants

Chromatogram:

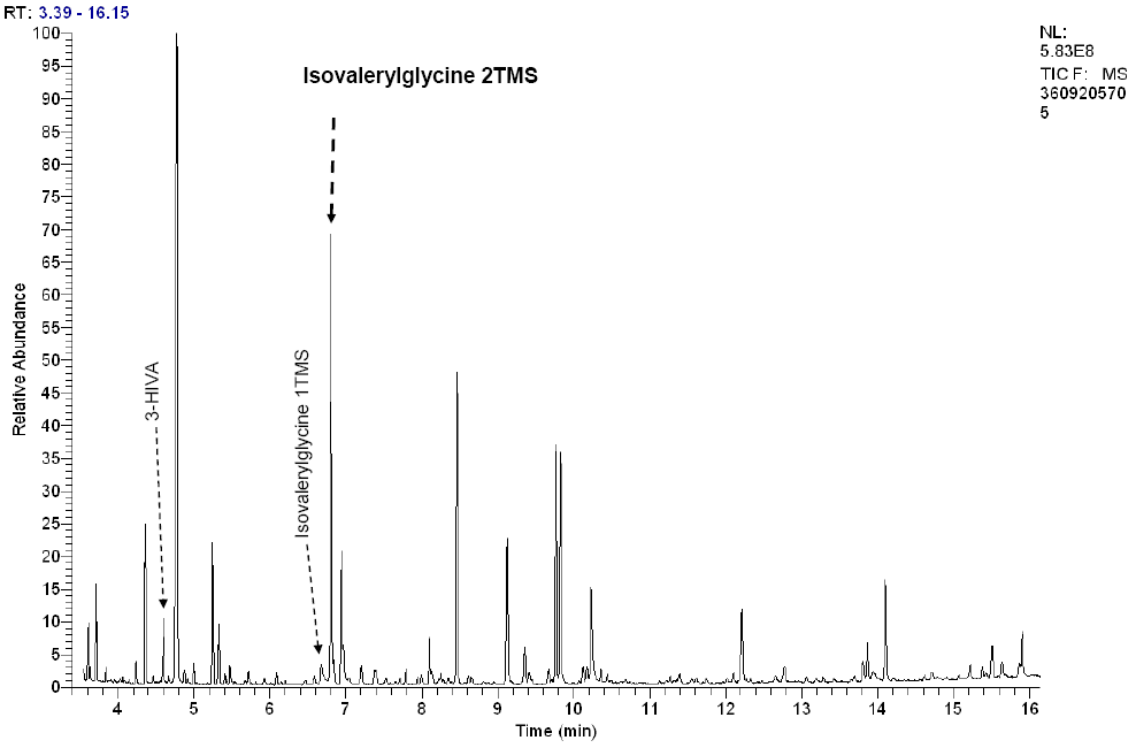

Fig 4: Organic acid profile of isovaleric aciduria

Sample 193:

Patient details: 5-year old boy with poor feeding, lethargy

Known diagnosis: Normal pattern

Diagnosis: Most of the participants (98%) reported a normal profile

Sample 194:

Patient details: 3-year-old girl with muscle hypotonia and seizures

Known diagnosis: 3-methylcrotonyl-CoA carboxylase deficiency

Analytical details:

3-methylcrotonylglycine is elevated. With trimethylsilylation the di TMS derivative is the most prominent signal.

3-methylcrotonylglycine was reported by 99% of the participants.

Diagnosis:

3-methylcrotonyl-CoA carboxylase deficiency or 3-methylcrotonylglycinuria was diagnosed by 93% and multiple carboxylase deficiency (biotinidase, holocarboxylase synthetase) by 4% of the participants.

Chromatogram:

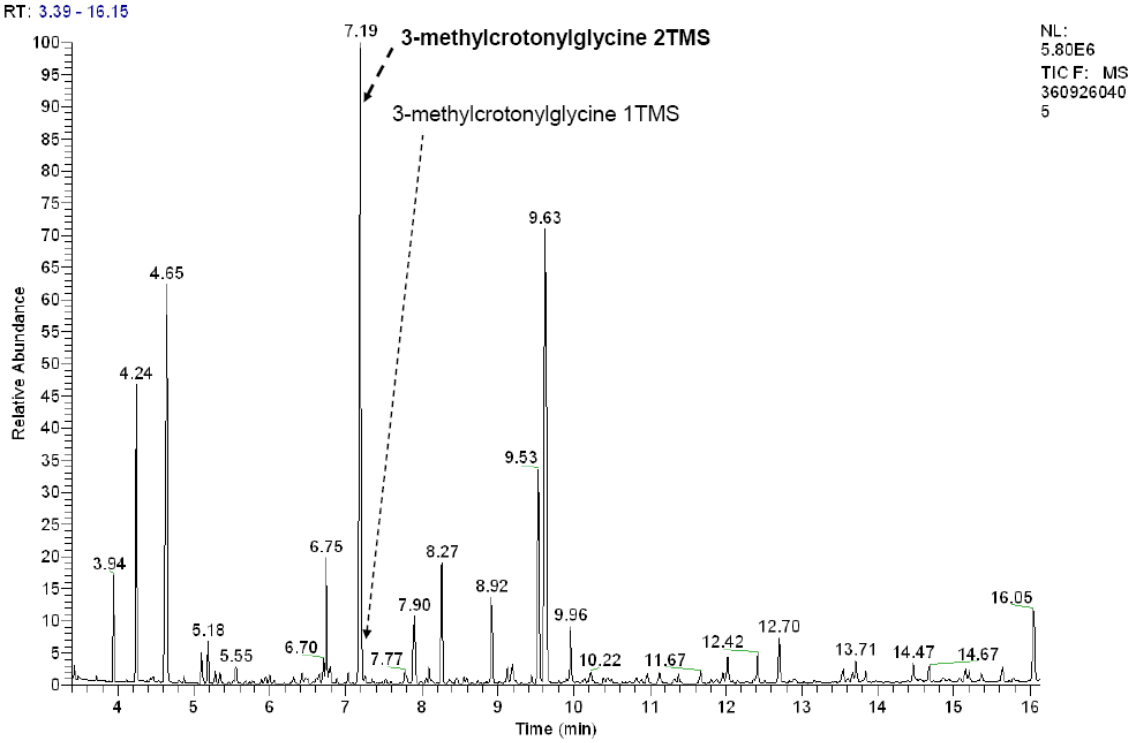

Fig 5: Organic acid profile of 3-methylcrotonyl-CoA carboxylase deficiency

Sample 195:

Patient details: 8-month-old boy after start of medication. At age 4 months rickets, nephromegaly and liver dysfunction

Known diagnosis: Tyrosinaemia type I

Analytical details:

Clearly detectable are the phenolic acids 4-hydroxyphenylacetic acid, 4-hydroxyphenyllactic acid and 4-hydroxyphenylpyruvic acid, the latter as two peaks for the syn and anti form. These were reported by 76%, 96% and 74% respectively.

The excreted amount of the pathognomonic metabolite succinylacetone was low in this sample as a result of the medication. Succinylacetone appears as a characteristic group of four peaks after oximation with pentafluorobenzylhydroxylamine and trimethylsilylation.

Only 55% of the laboratories detected this metabolite.

Also detectable is N-acetyltyrosine.

Secondary findings are increased lactic acid and two peaks for chloralhydrate metabolites.

Diagnosis:

Tyrosinaemia type I was diagnosed by 88% of the participants

Chromatogram:

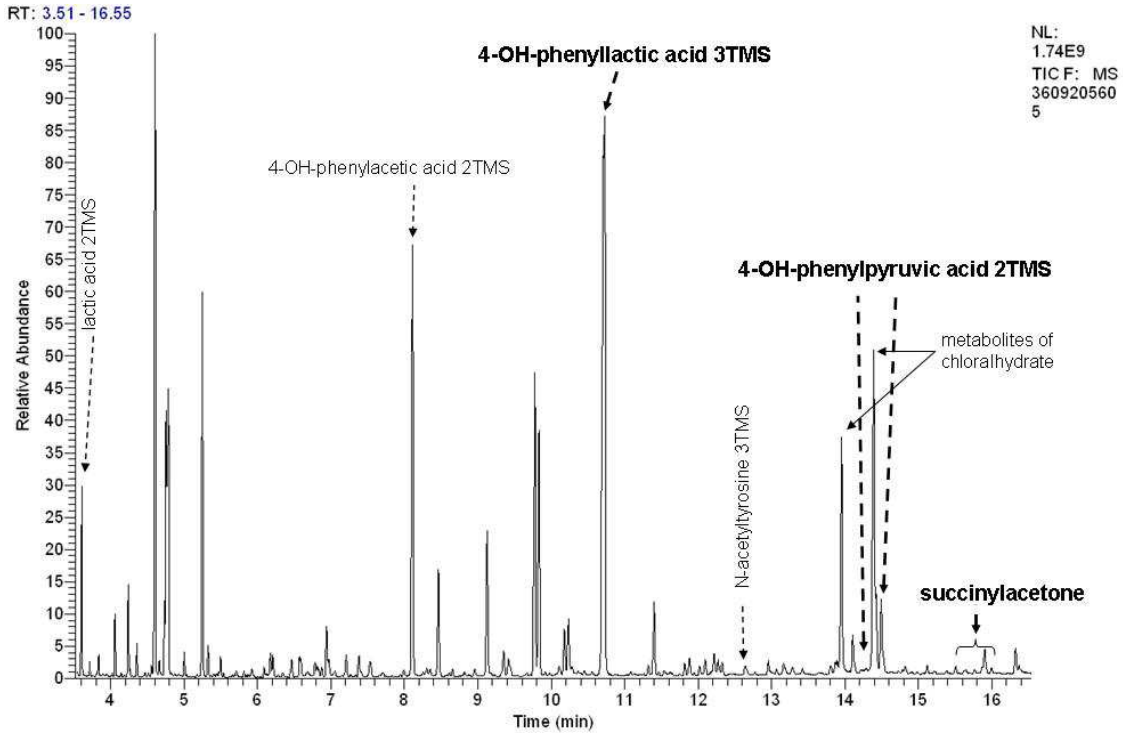

Fig 6: Organic acid profile of tyrosinaemia type I

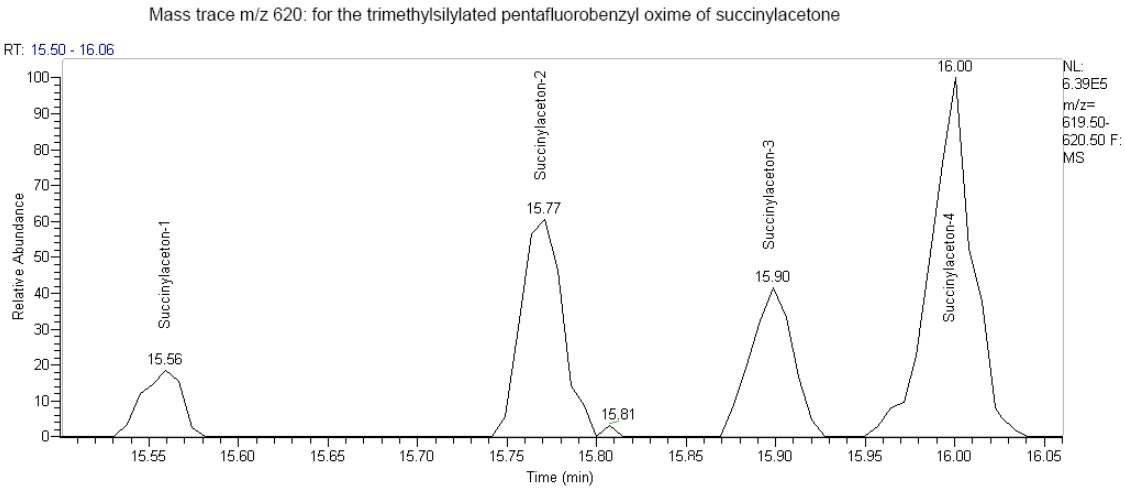

Fig 7: Mass fragmentograms of succinylacetone

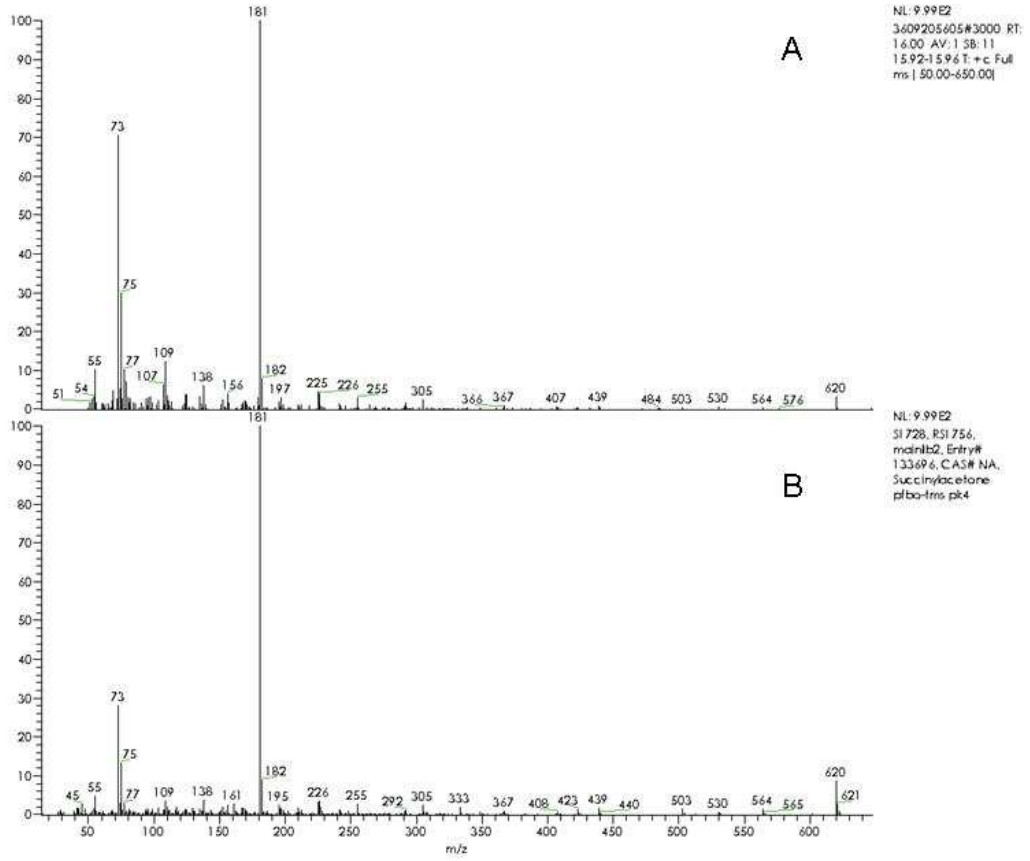

Fig. 8: Comparison of measured mass spectrum (A) and database mass spectrum (B) for the trimethylsilylated pentafluorobenzyl oxime of succinylacetone.

The participants´ cumulative scores are shown in table 5 and in figure 9.
Cumulative scores are the scores for the whole year. 2011 sixty-two participants (68.8%) got full marks!

Table 5: Cumulative total scores 2011 – 2005

	Number of laboratories						
Cumulative scores	**2011**	**2010**	**2009**	**2008**	**2007**	**2006**	**2005**
18	62	46	27	21	55	16	25
17	10	2	3	26	4	10	4
16	4	4	2	5	-	10	12
15				1	-	4	1
14	4	19	31	11	2	12	6
13			1	2	-	2	2
12	4	3	6	6	7	5	6
11	1			3	2	-	1
10	1	3	1	1	1	4	3
9		2	1	-	-	1	1
8	1	3	4	1	1	-	-
7	1			1	-	-	1
6	1		2	1	3	3	-
5				-	-	-	-
4		3	1	-	-	-	-
3				-	-	-	-
2				-	-	-	2
1				-	-	-	-
0	1	2	6	4	3	4	3

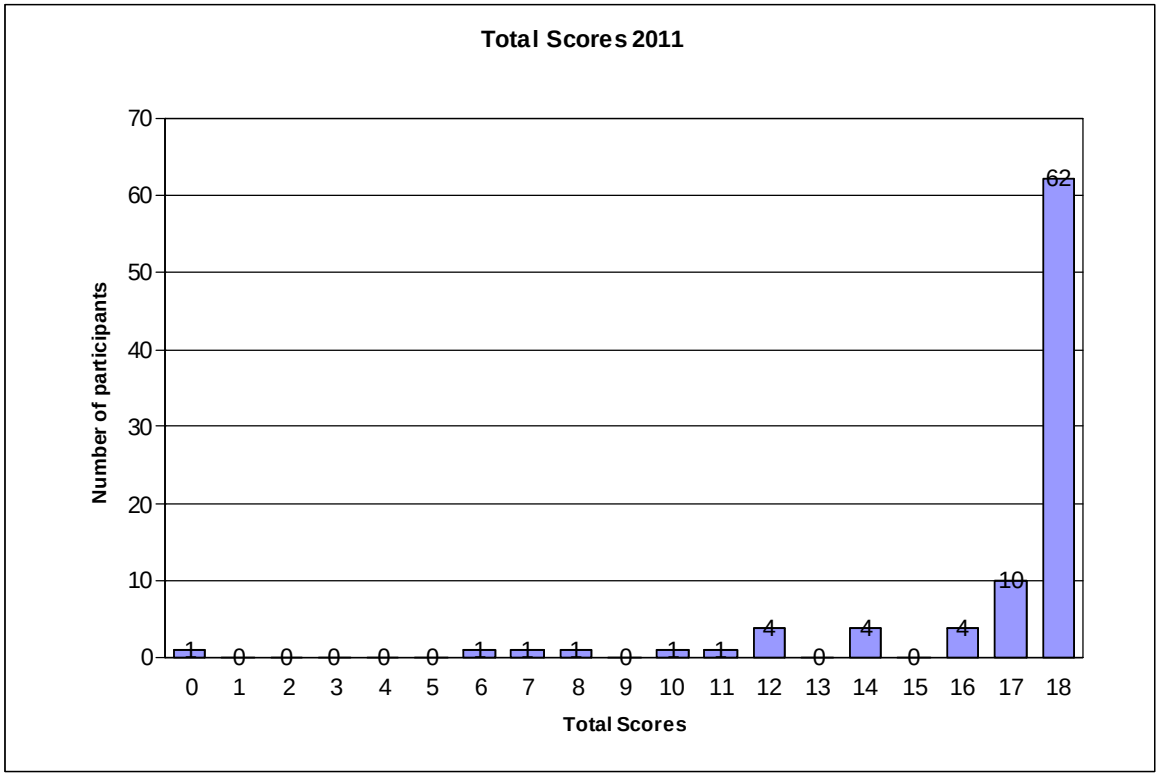

Fig. 9: Cumulative scores 2011

Your total score 2011

Your total score for 2011 was:

Your number of returns in 2011 was:

General comments

We would just like to point out here that we are not able to accept returns sent in after the report for the corresponding circulation has been mailed because this would not be compatible with the overall intention of the scheme. We are conscious of the fact that posted results could get lost on a variety of ways. Therefore it would be a good advice to send in results by more than one route (e.g. FAX and email, regular mail and FAX or email).

Special thank for the laboratories that supported us last year with samples. This is critical for the success of the program and will keep the scheme interesting. **It is most appreciated that you will continue to support us with urine from patients. Please send us at least 250 ml urine of any interesting patients you may have. We will cover the costs.**

Yours sincerely,

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Dr. V. Peters

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Prof. Dr. G. F. Hoffmann

Director
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